Table 1 *Statistical data on the effect of water hyacinth extract against the fifth instar larvae of C. cephalonica*

Dose (μg)/per cent inhibition*	Regression equation	χ^2 (μg)	$ID_{50} \pm S.E.$ (μg)	Fiducial limits (μg)
1.50/90 1.00/70 0.75/55; 0.50/38; 0.25/25	$1.16 \times 3.51X$	3.51	0.644 ± 0.09	0.825 0.463

* Inhibitory activity was rated as: Score 0: formation of normal insect that survived (perfect adult); Score 1: Adult with deformed wings that died 2–3 days after emergence; Score 2: Abnormal pupae; Score 3: Larval-pupal mosaics; Score 4: Death of the larvae in pre-ecdysial stages with blackened cuticle.

was calculated which was found to be 0.644 $\mu g \pm 0.09$ (table 1).

Preliminary chemical analysis reveals that the extract contains sterols which may be considered to be the main cause of these moulting abnormalities. From the present study, it can be concluded that the extract has the potentiality to disrupt the growth and reproduction of *C. cephalonica*. Studies are in progress to identify the chemistry of the active principle.

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FORMATION OF APOTHECIA BY SCLEROTIA OF *SCLEROTINIA TRIFOLIORUM* ERIKSS — A NEW RECORD IN INDIA

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STEM-ROT of Berseem (*Trifolium alexandrinum* L.) caused by *Sclerotinia trifoliorum* is one of the most serious diseases of this forage legume in Punjab. The disease was extensively studied in Europe, Northern USA and Canada on perennial clovers, alfalfa and other legumes[1]. Recently the disease was reported on Berseem[4] but no information is available on the formation of apothecia by sclerotia of *S. trifoliorum* from India. Sclerotia germinate in the field either myceliogenically or carpogenically and cause infection but the secondary spread of the disease is through mycelium[3,5].

The main objective of this study was to determine the time and depth at which the sclerotia of *S. trifoliorum* germinate to form apothecia.

Sclerotia of *S. trifoliorum* were collected from the naturally infected fields of Berseem in June 1986, air-dried and stored at 25–40°C. Sclerotia were also obtained from the culture of *S. trifoliorum* grown for one month on potato dextrose agar and stored.

Earthen pots were filled with sterilized sandy loam soil. The sclerotia collected from field as well as from culture were buried at different depths of soil (0, 0.5, 1, 2, 3, 4 and 5 cm). Ten sclerotia were added to each pot in three replicates during the last week of October and Berseem variety BL-1 was sown in these pots. The seedlings were established by sprinkler irrigation. The pots were examined weekly for the production of apothecia. Only open,

Figure 1. Apothecia developed from sclerotia buried 1 cm deep in the soil.

Table 1 *Effect of burial of sclerotia at different depths in soil on the formation of apothecia*

Depth (cm)	Number of apothecia observed* December 1986**	January 1987**	February 1987**	Mean
0.0	–	–	–	–
0.5	14	18	5	12.3
1.0	23	19	13	18.3
2.0	15	13	21	16.3
3.0	9	12	14	12.3
4.0	–	3	–	1.0
5.0	–	–	–	–

– Apothecia did not form; * Average of 3 replicates containing 10 sclerotia each; ** Number of apothecia (total of 4 weeks).

erect and live-appearing apothecia were counted.

The results indicate that numerous apothecia developed in December (figure 1) from field collected sclerotia buried at 1 cm followed by 2 cm depth. However; the maximum number of apothecia developed in February at 2 cm depth. A few apothecia developed in December from sclerotia buried at 3 cm depth, but their number increased in January and February. Very few apothecia developed during January from sclerotia buried at 4 cm depth. Sclerotia buried at 5 cm depth did not produce apothecia even up to February (table 1). Apothecia were also not formed from sclerotia produced in culture.

Burial of sclerotia at >3 cm depth greatly reduced the number of apothecia. The formation of apothecia was abundant as early as December and ceased by March. These findings were supported by earlier workers[2,6]. Varying number of apothecia at different depths indicates the possibility of apothecial production in the next season from sclerotia buried up to 5 cm depth when they come up with the tillage practices.

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A MODIFIED TECHNIQUE FOR DIFFERENTIAL STAINING OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS AND ROOT TISSUES

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SYMBIOTIC association of vesicular-arbuscular mycorrhizal (VAM) fungi with plant roots has drawn considerable attention in the recent past. The most commonly used method for the study of mode of infection and the type and degree of root colonization by the VAM fungi is the staining of these fungi in association with the roots. Several stains viz. cotton blue, Sudan IV[1], Trypan blue[2] etc. have been tried but acid fuchsin[3] is reported to be most satisfactory. It stains fungal structures dark red against light to medium red root tissues background. A double staining (toluidine blue-O-acid fuchsin) system, which gives differential colour with root tissues and fungal structures, was developed during the present study and this is found to be superior to the acid fuchsin method.

In order to obtain roots colonized by the mycorrhiza, surface-sterilized (2 min dipping in 1:1000 W/V $HgCl_2$; 4 washings in sterilized distilled water), mungbean (*Vigna radiata* L.) seeds were sown in plastic pots containing sterilized sand mixed with chlamydospores of *Glomus mosseae* (Nicol. & Gerd.) Gerdemann and Trappe comb. nov. and incubated at 25 ± 3°C. Hoagland's solution[4] with half level of phosphorus was periodically applied as a source of nutrients. Two-month-old mungbean plants were uprooted and their roots were washed carefully under running tapwater. The method described by Phillips and Hayman[5] was followed for clearing of roots. Roots (1 cm) were boiled in 10% KOH at 90°C for 45 min, rinsed with distilled water and acidified with dilute HCl. For staining, acidified roots were washed with distilled water, dipped in aqueous solution of toluidine blue-O (0.1%) for 5 min, dehydrated through alcohol series, stained in acid fuchsin for 10 min, cleared in xylene and mounted in D.P.X. mountant.

As a result of the proposed staining procedure, the fungal mycelium attained medium red colour,